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Community composition modifies direct and indirect effects of pesticides in freshwater food webs

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Abstract

For environmental risk assessment, the effects of pesticides on aquatic ecosystems are often assessed based on single species tests, disregarding the potential influence of community composition. We, therefore, studied the influence of changing the horizontal (the number of species within trophic levels) and vertical composition (number of trophic levels) on the ecological effects of the herbicide linuron and the insecticide chlorpyrifos, targeting producers and herbivores, respectively. We tested how adding, to a single primary producer, 4 selected competing producer species, 0-1-4 selected herbivore species, and one selected predator species resulting in 1, 2 and 3 trophic levels, changes the effects of the two pesticides.

Linuron decreased producer biovolume less (17%) when the 4 producers were added, because insensitive producers compensated for the loss of sensitive producers. However, linuron decreased producer biovolume 42% and 32% more as we increased the number of

herbivore species from 0 to 4 and as we increased trophic levels from 1 to 3, respectively. The indirect negative effect of linuron on herbivore biovolume was 11% and 15% lower when more producer and herbivores were added, respectively. Adding a predator increased this indirect negative effect by 22%.

Chlorpyrifos decreased herbivore biovolume about 10% less when adding multiple herbivore or producer species. However, adding a predator magnified the direct negative impact on herbivores (13%). Increasing the number of producer, herbivore species and adding trophic levels increased the indirect positive impact on producer biovolume (between 10% and 35%).

Our study shows that changing horizontal composition can both increase and decrease the effects of the selected pesticides, while changing vertical composition by adding number of trophic levels always increased these effects. Therefore, single species sensitivity will not always represent a worst case estimate of ecological effects. Protecting the most sensitive species may not ensure protection of ecosystems.

Keywords:

Producers, Herbivores, Vertical composition, Linuron, Chlorpyrifos

1. Introduction

Ecological risk assessment of chemicals is mainly based on the results of single-species laboratory tests performed with algae, daphnia and fish, representing a limited set of standard test species (Artigas et al., 2012; Brock et al., 2006; Rohr et al., 2016). However, community composition in natural ecosystems often is more complex and how to address this difference

in community composition is considered one of the most difficult challenges in ecotoxicology (De Laender and Janssen, 2013; Rohr et al., 2016; Van den Brink et al., 2018). Community composition in natural systems can be characterised in two dimensions: the number of species within trophic levels (horizontal composition) and number of trophic levels (vertical composition). Both dimensions of composition could influence the effects of chemicals on aquatic communities (Baert et al., 2016; De Laender et al., 2015; Zhao et al., 2019). Recent work showed that the two dimensions had contrasting effects on the short-term stability of whole food webs (using total biomass as a proxy) after pesticide exposure (Zhao et al., 2019). However, how the two dimensions influence direct and indirect effects of chemicals after prolonged exposure is at present unknown.

The direct negative effects of herbicides on population size of primary producers (hereafter named 'producers') can be smaller when more producer species are added (Baert et al., 2016). A more diverse producers' community can include both sensitive and tolerant producers (Baert et al., 2016). When environmental stressors reduce the population of sensitive producers, negative interactions among producers result in competitive release, so that reductions in populations of sensitive species can be compensated by an increase of tolerant species (Baert et al., 2016; De Laender et al., 2016; Gonzalez and Loreau, 2009). In contrast, the direct negative effects of herbicides on producer populations can be larger as more herbivore species are added, because herbicides and herbivore grazing could interact to aggravate the herbicide effects (Halstead et al., 2014; Rohr et al., 2006; Rohr and Crumrine, 2005). Conversely, the presence of a predator could suppress the herbivore population (Anderson et al., 1996; Pace et al., 1999), and the resulting decrease in grazing pressure could alleviate the direct negative effects of herbicides on producers.

69 The direct negative effects of insecticides on herbivores (population size) can be smaller as
70 more herbivore species are added, again due to compensation. A more diverse herbivores'
71 community can include both sensitive and tolerant herbivores, while more intolerant
72 herbivores have larger probability to be included (Becker and Liess, 2017). Insecticides
73 decrease the populations of sensitive herbivores, resulting in its resource (producers) being
74 released from grazing, which in turn can result in an increase of tolerant herbivores via an
75 increase of food resources (Rohr and Crumrine, 2005). The indirect benefit of insecticides on
76 tolerant herbivores can thus compensate the decline of sensitive herbivores . The insecticide
77 can also be hypothesized to affect herbivores less when more producer species are added,
78 because of an increased probability that an edible producer would occur that promotes
79 herbivore growth (Haddad et al., 2011). However, the insecticide could affect herbivore
80 population size more severely when a predator is present, because of synergistic interactions
81 between the insecticide and predation (Beketov and Liess, 2006; Relyea and Mills, 2001;
82 Trekels et al., 2013). For example, Relyea and Mills (2001) reported that the pesticide carbaryl
83 was 4 times more toxic to the prey (tadpoles) when a predator (*Ambystoma maculatum*) was
84 present. Some studies, however, showed that interactions between insecticides and presence
85 of a predator on herbivores can be additive or antagonistic (Campero et al., 2007; Janssens
86 and Stoks, 2017, 2013; Trekels et al., 2011).

87 The indirect effects of pesticides are also expected to depend on horizontal and vertical
88 composition. Herbicides could indirectly decrease herbivore population size, due to a
89 decrease in edible producer biomass (Bracewell et al., 2019; Fleeger et al., 2003; Preston,
90 2002). We expect that the herbicides could decrease herbivores even more when a predator
91 is present, due to an increase of both bottom-up and top-down control (Clements and Rohr,
92 2009; Rohr et al., 2006; Rohr and Crumrine, 2005). In addition, insecticides could, indirectly,

induce an increase of producer population size, because of the top-down induced release of producers (Clements and Rohr, 2009; Halstead et al., 2014; Rohr et al., 2006; Rohr and Crumrine, 2005). It is thus expected that the release of producers could be stronger when a predator is present as this will serve as an extra top-down effect.

To test these hypotheses, we conducted microcosm experiments mimicking planktonic food webs in which we added 4 selected competing producer species to a single producer, 0, 1 or 4 selected herbivore species, and one selected predator species. By doing so we also changed vertical composition (1, 2 and 3 trophic levels). We then tested whether horizontal and vertical composition influences either the effects of the herbicide linuron or the effect of insecticide chlorpyrifos

2. Materials and Methods

2.1. Experimental conditions

We experimentally tested the effect of horizontal and vertical composition on simple food webs exposed to pesticides. The experiments, which lasted for 21 days, were performed in 900 mL glass jars, filled with 500 ml WC medium and contained in a water bath at constant temperature (19.9 °C ± 0.8 °C) and a light regime of 12h: 12h (light: dark). The light intensity at the surface (measured with a LI-COR LI-250A, LI-COR Biosciences, Lincoln, USA) was 120 µmol m^{-2} s^{-1}, and was created using Ceramalux® Phillips 430 Watt High Pressure Sodium Non-Cycling Lamps.

2.2. Organisms

We obtained all producers and herbivores from cultures present at the Aquatic Ecology and Water Quality Management group of Wageningen University. Five green alga (*Scenedesmus acutus, Chlorella vulgaris, Desmodesmus pannonicus, Raphidocelis subcapitata* and *Scenedesmus obliquus*) were randomly selected as producers while four cladoceran (*Daphnia magna*, *Daphnia pulex*, *Daphnia lumholtzi* and *Moina macrocopa*) species were randomly selected as herbivores. All these organisms were collected from Dutch lakes or ditches and then cultivated in the lab. The algae were cultured in WC medium under continuous light. The herbivores were cultured in RT medium using a natural day/night light rhythm (Tollrian, 1993) and fed with algae *C. vulgaris* at 10^{-5} cell ml^{-1} day^{-1}. One individual of *Chaoborus obscuripes* was selected as a predator. *C. obscuripes* was collected from Sinderhoeve Experimental Station (www.sinderhoeve.org; Renkum, The Netherlands). Before addition, *C. obscuripes* was kept in a 5 L plastic bucket with 1.5 L pond water and 1.5 L WC medium, stored in a fridge (4-7 °C) to slower the moulting and fed with cladocerans every three days. Before the experiments started, herbivores and predators were separately moved into WC medium to starve for 24 h, so that their guts were cleared of pre-fed food.

2.3. Experimental setup

To a single randomly selected primary producer (*R. subcapitata*), we added 4 producers, 0, 1, or 4 selected herbivores, and one selected predator resulting in 1, 2 and 3 trophic levels (vertical composition). The other four producers were *S. acutus, C. vulgaris, D. pannonicus* and *S. obliquus*. The single herbivore was *M. macrocopa* (randomly assigned). The other three herbivores were *D. magna, D. pulex,* and *D. lumholtzi*. The predator was *C. obscuripes*.

We adopted a design where we manipulated horizontal and vertical composition, as well as the exposure to contaminants. To manipulate composition, we crossed horizontal

137 composition of the producers (two levels; 1 or 5 species) and horizontal composition of the
138 herbivores (three levels; 0, 1 or 4 species), resulting in 6 food-web structures. When
139 consumers were present, we also manipulated the presence of a predator (absent or present),
140 resulting in 4 more food-web structures. We therefore also manipulated vertical composition
141 (1, 2 and 3 trophic levels). This gives a total of 10 different food-web structures (Table S1). To
142 manipulate exposure to contaminants, we either exposed these compositions to the
143 insecticide chlorpyrifos (0 and 1 µg l^{-1}), or the herbicide linuron (0 and 100 µg l^{-1}). The 0 µg l^{-1}
144 linuron and chlorpyrifos treatments served as controls. The nominal concentration of 100 µg
145 l^{-1} linuron was chosen because it is higher than the 72d EC_{50} for relative growth inhibition of 6
146 µg l^{-1} for *Scenedesmus acutus* (Snel et al., 1998) and lower than the 21 days NOEC value (180
147 µg l^{-1}) for reproduction of *D. magna* (Crane et al., 2007). It was expected that the
148 concentration had no direct toxic effect on herbivores but only on producers (Cuppen et al.,
149 1997; Slijkerman et al., 2005). The nominal chlorpyrifos concentration of 1 µg l^{-1} is the 48h LC_{50}
150 value for *D. magna* (Kersting and van Wijngaarden, 1992), so that treatment effects were
151 supposed to not completely eliminate the herbivores and allow recovery (Daam et al., 2008;
152 Van den Brink et al., 1996).

153 We replicated each treatment four times, leading to 10 food web structures x 2 contaminants
154 x 2 treatments (control and contaminant treatment) x 4 replicates = 160 vessels in total. The
155 initial total biovolume of producer and herbivores was always 25 mm^3 and 0.2 mm^3,
156 respectively, regardless of producer and herbivores richness. For the systems with all three
157 trophic levels, we added one individual of the predator *C. obscuripes* to each system. We made
158 sure the predators used in the experiments had a mean (±SD) individual body length of 10.46
159 ± 0.11 mm to avoid a bias introduced by body size-dependent feeding rates.

160 *2.4. Chemical application and analysis*

161 All stock solutions for linuron and chlorpyrifos were created in a same way that 5 mL of stock
162 solution was diluted with WC medium to reach the desired concentration. For the stock
163 solution of linuron, we diluted the commercial product Afalon® Flow with a linuron
164 concentration of 450 mg ml^{-1} to 10 µg ml^{-1}. The stock solution of chlorpyrifos was achieved by
165 diluting a commercial formulation Dursban® 4E, with a chlorpyrifos concentration of 480 mg
166 ml^{-1} to 0.1 µg ml^{-1}. Then 5 mL of stock solution was added into the system. Each system was
167 filled WC medium up to 500 ml and stirred 15 seconds immediately before the start of the
168 experiment.

169 To monitor the chemical degradation during the experiment samples were taken after 1h, 2,
170 4, 6, 14 and 21 days of exposure. In order to analytically verify the linuron concentration of
171 each experimental jar, 2 mL of water sample was added to 0.5 ml methanol. The chemical
172 concentration was analysed according to Van den Brink et al. (1997), through Agilent
173 Technologies LC-QQQ Mass spectrometer with a binary pump, Bin Pump, model G1312A, with
174 MilliQ + 0.1% fatty acid as solvent A and methanol + 0.1 % fatty acid as solvent B with a ratio
175 of 20:80. For the chlorpyrifos analysis, 8 mL samples were taken from each system and then 2
176 ml *n*-hexane was added, followed by vortex for 1 minute under 1000 revolution per second. A
177 1mL subsample was transferred to a GC vial, then followed by GC and electron capture
178 detection to determine the exact concentration of chlorpyrifos (Rubach et al., 2011).

179 *2.5. Ecological endpoints*

180 We estimated the biovolume and composition of producers and herbivores, and biovolume of
181 predators in each replicate on day 2, 4, 6, 14 and 21 day after the beginning of the experiment.
182 At each sampling day, we first sampled the controls followed by the exposure jars to prevent

183 cross contamination. Producer biovolume ($mm^3\ l^{-1}$) was measured with a CASY® Cell Counter
184 model TT (innovates AG CASY®- Technology). In order to estimate algae composition, 900 µl
185 algae samples were stained with 100 µl lugol preservative for microscopic enumeration of
186 algal cells using an inverted light-microscope (Nikon Eclipse E100 microscope with a DS-2Mv-
187 L2 camera; Nikon Corp., Tokyo, Japan) at 200 magnification. Herbivore biovolume ($mm^3\ l^{-1}$) in
188 each replicate was calculated as abundance (individual l^{-1}) times individual biovolume (mm^3
189 $individual^{-1}$). The individual biovolume (mm^3 $individual^{-1}$) of herbivores and predators were
190 measured by a formula $0.074 * L^{2.92}$ (L is length in mm) (Horn, 1991), where the body length
191 was estimated using light microscopy Olympus szx10 (Olympus Corp, Tokyo, Japan) at 10
192 magnification. Abundance and composition was recorded after sucking all individuals into an
193 inverted 10 mL serological pipette to put into 50 ml culture dish that filled with 20 ml WC
194 medium. Afterwards herbivores and predators were put back in their beakers for next
195 sampling.

196 *2.6. Data analyses*

197 Biovolume or abundance were used to calculate the effect sizes for the producers, herbivores
198 and the predator, while chlorophyll *a* was used to compute effect size for photosynthetic
199 capacity. Effects sizes were calculated by dividing the value for the treatment by the mean of
200 control so that an effect size smaller than 1 indicates a negative impact of the chemical on the
201 producers, herbivores or the predator, a 1 no effect, while effect sizes larger than 1 indicates
202 a positive impact. To each of chemicals, three-way ANOVA's were used to estimate the effects
203 of the horizontal composition of producers and herbivores, the vertical composition and with
204 all combination of interactions on the effect sizes of producers (abundance, biovolume,
205 chlorophyll *a*) and herbivores (abundance or biovolume) on sampling days 2, 4, 6, 14 and 21,

206 respectively, yielding 50 three-way ANOVA's (5 response variables × 5 sample days × 2
207 pesticides). We adopted the same approach for the effect sizes of predator biovolume.
208 However, note that by definition, in the case of the presence of a predator, the vertical
209 composition was always three, so we could only analyse the effects of horizontal composition,
210 yielding 10 two-way ANOVA's (1 response variables × 5 sample days × 2 pesticides). Normality
211 of model residuals was verified by the QQ-plot (Fig. S1–S2).

212 The effects of herbicide (insecticide) on producers (herbivores) were the largest on day 6 (see
213 results section). We used raw data (biovolume, density or chlorophyll *a*) on this day to
214 understand the interactions between treatments. The raw data were natural log-transformed
215 prior to analysis. For each pesticide data set, we applied four-way ANOVA's to estimate the
216 effect of horizontal composition of producers and herbivores, vertical composition, pesticide
217 and their pairwise interactions, on (1) producers (abundance, biovolume and chlorophyll *a*)
218 and (2) herbivores (abundance and biovolume), respectively, yielding 10 four-way ANOVA's (5
219 response variables × 2 pesticides). Normality of model residuals was verified by the QQ-plot
220 (Fig. S3–S4). We adopted the same statistical approach for the effect on predator biovolume.
221 However, note that by definition, vertical composition is always three when a predator is
222 present, so we could only analyse the effects of horizontal composition, yielding 2 three-way
223 ANOVA's (1 response variable × 2 pesticides). Normality of model residuals was again verified
224 by the quantile–quantile (QQ) plot (Fig. S3–S4). Finally, to evaluate the effects on community
225 composition on day 6, the day with the maximum effects, we again used four-way ANOVA's
226 to test the effect of horizontal composition of producers, herbivores and vertical composition,
227 pesticide and their pairwise interactions, on ln(biovolume) of (1) the producer species (*R.*
228 *subcapitata,* i.e. the single producer treatment) and (2) the herbivore species (*M. macrocopa,*
229 i.e. the single herbivore treatment). For the other four producer species (*S. acutus, C. vulgaris,*

D. pannonicus and *S. obliquus*), a three-way ANOVA's was used to test the effect of horizontal composition of herbivores, vertical composition, pesticides and their pairwise interactions on ln(biovolume), because the horizontal composition of producers was always five. Similarly, we used three-way ANOVA's to test the effect of horizontal composition of producers, vertical composition, pesticide and their pairwise interactions on ln(biovolume) of each of the rest three herbivore species (*D. magna, D. pulex*, and *D. lumholtzi*), because the horizontal composition of herbivores was always four. The analysis of community composition yielded 18 ANOVA's (9 response variables × 2 pesticides). Normality of model residuals was again verified by the quantile–quantile (QQ) plot (Fig. S5–S6).

3. Results and discussion

3.1. Pesticide concentration

The mean start concentrations for linuron and chlorpyrifos in the experimental systems were 94.2 (± 8.4)% and 87.8 (± 9.4)% of the nominal concentrations, respectively (Fig 1). The dissipation half-life (DT50) for linuron could not be calculated (> 21 d; Fig 1), while the DT50 of chlorpyrifos was between 5-8 days. The observed persistence of linuron and chlorpyrifos were in line with those observed in other planktonic systems by Daam et al. (2008, 2009) and Daam and Van den Brink (2007) who reported DT50 values of > 21 days for linuron and 6-10 d for chlorpyrifos.

3.2. Effect of linuron

3.2.1 Influence of community composition on direct effects

Throughout experiments, the direct negative effect of the herbicide linuron on producer biovolume was, on average, 17% smaller when the 4 producer species were added (Fig. 2a). However, this direct negative effect was 42% larger when the number of herbivore species was increased from 0 to 4 and 32% larger when vertical composition was changed from 1 to 3 (Fig. 2b-c). On day 6, linuron had its maximum effect on producer biovolume (Fig. 2a-c). The negative effect of linuron on producer biovolume was larger when adding more herbivore species and when vertical composition was higher, regardless of the composition of the producer community (Fig. 3a-b). The negative effects were strongest when the number of producer species was lowest, the number of herbivore species highest, and vertical composition equal to 2 (Fig. 3a). These trends were similar when using chlorophyll *a* (photosynthetic capacity) as an endpoint (Fig S7. a-c; Fig S8. a-b).

Adding producers decreased the direct negative effect on producer biovolume (Fig. 2a) and chlorophyll *a* (Fig S7. a-c), due to the decrease of sensitive chlorophytes biovolume (e.g. *R. subcapitata and C. vulgaris*) compensated by other tolerant chlorophytes (e.g. *S. obliquus*) (Fig. 4a). *R. subcapitata*, previously known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata,* has a 5d EC_{50} of 67 µg l^{-1} based on abundance (USEPA, 2020), explaining its decrease in biovolume (Fig. 4a). *C. vulgaris* and to a lesser extent *S. acutus* also show a decrease in biovolume, which can be explained by their 7d EC_{50} of 50 µg l^{-1} also based on abundance and 3d EC_{50} of 8.9 µg l^{-1} based on population growth rate, respectively (Stephenson and Kane, 1984; USEPA, 2020). The other two species, *D. pannonicus* and *S. obliquus*, showed no response or an increase in biovolume (Fig. 4a) and, unfortunately, no sensitivity data is available, but *S. obliquus* became relatively abundant in small plankton dominated microcosms stressed by 150 µg l^{-1} linuron (Daam and Van den Brink, 2007). Some semi-field experiments also showed that linuron had both positive and negative effects on Chlorophytes

(Daam et al., 2009; Slijkerman et al., 2005; Van den Brink et al., 1997). For example, Daam et al. (2009) reported that some of chlorophytes (e.g., *Coelastrum cambricum* and *Pediastrum duplex*) decreased in population size, which was compensated by increases of other chlorophytes (e.g., *Ankistrodesmus falcatus*, *Oocystis pusilla* and *Oocystis lacustris*).

In contrast, the negative impact of linuron on producers was larger when more herbivores were added (Fig. 2b), due to a larger suppression of producer biovolume (e.g. *C. vulgaris*) (Fig. 4a) when multiple herbivores were present. Multiple herbivore species more effectively reduce producer population sizes than a single herbivore species because of larger consumption rates (Duffy et al., 2003; Naeem and Li, 1998).

The presence of a predator (*C. obscuripes*) decreased the biovolume of the herbivores (Fig. 3c-d; Fig. 4b), as has also been reported by Black and Dodson (1990) and Hebert and Grewe (1985), due to predation. The presence of the predator hence alleviated the grazing pressure on producers, which made the direct negative effect of linuron on producers smaller than the treatments with producers and herbivores only, i.e. vertical composition=2, (Fig. 2c). However, the presence of the predator did not eliminate all herbivores. Thus, the herbivores still consumed producers (e.g., especially small sized *C. vulgaris*) (Fig. 4a). Hence, the presence of a predator and herbivores still made the negative effect of linuron on producers larger than the negative effect of linuron on producers in treatments where only producers were present, i.e. when vertical composition was equal to 1, (Fig. 2c).

3.2.2 Influence of community composition on indirect effects

The herbicide-induced decrease of producers led to indirect negative effects on herbivore biovolume (Fig. 2d-f). Some semi-field experiments found both negative and positive impacts of linuron on herbivores (Cuppen et al., 1997; Daam et al., 2009). For example, Cuppen et al.

298 (1997) reported negative effects of linuron on Rotatoria but positive effects on Copepoda.
299 They attributed these negative and positive linuron effects to the preferred resources of these
300 herbivores, i.e. diatoms for Rotatoria and *Chlamydomonas* for Copepoda, respectively, the
301 latter showing a large increase in the linuron stressed systems (Van den Brink et al., 1997).
302 Here, we only found negative effects of linuron on herbivores, which can be attributed to
303 herbivores consuming all producer species and the overall decrease in biovolume of the algae
304 species (Fig. 4a) as no adaptation was found like as by Van den Brink et al. (1997).

305 In addition, the indirect negative effect of linuron on herbivore biovolume was 11% smaller
306 when 4 producers were added and 15% smaller when the number of herbivore species was
307 increased from 1 to 4 (Fig. 2d-e). This was because adding more producers and herbivores
308 caused an increase of the absolute biovolume of herbivores on day 6 (Fig. 4b). However, the
309 indirect negative effect of linuron on herbivores was 22% larger when vertical composition
310 was changed from 2 to 3 (Fig. 2f), because predation decreased the absolute biovolume of
311 each herbivore species (Fig. 4b). On day 6, linuron also had its maximum effect on herbivore
312 biovolume (Fig. 2d-f). The negative effect of linuron on herbivore biovolume was smaller when
313 more producers and herbivores were present, independent of vertical composition (Fig 3c-d).
314 The negative effects were smallest when the number of producer species was highest, the
315 number of herbivore species highest and the vertical composition equal to 2 (Fig 3c). We
316 detected qualitatively identical results (single and interactive effects) using abundance as a
317 proxy (Fig S7d–7i; Fig. S8), even though the magnitude of decreases and increases was smaller.
318 We did not detect significant effect of composition on the predator biovolume (Table S2).

319 *3.3. Effect of chlorpyrifos*

320 *3.3.1 Influence of community composition on direct effects*

As found for linuron, the direct effect of chlorpyrifos also depended on horizontal and vertical composition. The direct negative effect on herbivore biovolume was, on average, 7% smaller when the number of herbivore species was increased from 1 to 4 and 12% smaller when 4 producers were added, while the negative direct effect was 13% larger when vertical composition was changed by adding a predator (Fig. 5a-c). On day 6, chlorpyrifos also had its maximum effect on herbivore biovolume (Fig. 5a-c). The negative effect of chlorpyrifos on herbivore biovolume was smaller with adding more producers and more herbivores across any level of vertical composition (Fig 6a-b). The negative effects were smallest when the number of producer species was highest, the number of herbivore species highest, and the vertical composition equal to 2 (Fig 6a).

The negative direct effect of chlorpyrifos on herbivores was smaller when adding more herbivores (Fig. 5b), which was associated with the loss of sensitive herbivores (e.g. *M. macrocopa*, *D. magna* and *D. pulex*) being compensated by other more tolerant herbivores (e.g. *D. lumholtzi*) (Fig, 7a). Only for *D. magna*, *M. macrocopa* and *D. pulex*, single species toxicity values could be found with 2-6d EC_{50} values of 0.20, 0.27 and 0.21 µg l^{-1}, respectively (Na et al., 2012; USEPA, 2020), explaining their decrease. The compensation of sensitive species by more tolerant ones has been shown previously (Daam et al., 2008; Van Wijngaarden et al., 2005). For example, Daam et al. (2008) showed that the decrease of Cladocera (e.g. *Streblocerus pygmaeus*) by chlorpyrifos was compensated by other tolerant Cladocera (e.g. *Dunhevedia crassa*).

In addition, we found that the direct negative effect of chlorpyrifos on the herbivore population was smaller when adding more of its resource (i.e. producer) (Fig. 5a), due to higher producers increasing the biovolumes of herbivores (e.g. *D. lumholtzi*) (Fig. 7a). However,

the presence of a predator *C. obscuripes* made the negative effect of chlorpyrifos on herbivores larger (Fig. 5c; Fig. 6a-b), as expected (Relyea and Mills, 2001; Van den Brink et al., 2017). For example, Relyea and Mills (2001) showed that, if a predator (*Ambystoma maculatum*) was present, the pesticide carbaryl was 4 times more toxic to the prey (tadpoles). Predation and chlorpyrifos has similar effects, and can produce synergistic effects when combined (Relyea and Mills, 2001).

3.3.2 Influence of community composition on indirect effects

The chlorpyrifos-induced decrease of herbivores resulted in indirect positive effects on producer biovolume (Fig. 5d-f), as found by Daam and Van den Brink (2007). This indirect positive effect was 10% stronger when 4 producers were added (10% stronger), the number of herbivore species was increased from 1 to 4 (35% stronger) and vertical composition was changed from 1 to 3 (33% stronger) (Fig. 5d-f). On day 6, chlorpyrifos also had a maximum effect on producer biovolume (Fig. 5d-f). The positive effect of chlorpyrifos on producer biovolume was highest when the number of producer species was highest, more trophic levels were present and the number of herbivore species equal to 1 (Fig 6c-d). The indirect positive effect on chlorophyll *a* was qualitatively similar (Fig. S9a-9c; Fig. S10a-b), but no significant effect of composition on predator biovolume was detected (Table S3). Again, we found qualitatively identical results (single and interactive effects) using abundance as proxy (Fig. S9d–9i; Fig. S10c-f).

The positive effect on producer biovolume (and chlorophyll *a*) can be understood from the release of grazing. The decrease of herbivores especially promoted the growth of its producer food source (e.g. *D. pannonicus*) (Fig. 7b). The increase was reinforced by adding producers, herbivores and trophic levels (Fig. 7b), making the positive effect on producer biovolume (and

chlorophyll *a*) larger (Fig. 5d-f; Fig. S9). Previous studies only reported chlorpyrifos-induced increase of producers (Daam et al., 2008; Daam and Van den Brink, 2007). For example, Daam and Van den Brink (2007) showed that chlorpyrifos application decreased herbivore abundances (Cladocera) and consequently caused an increase in chlorophyll *a* levels. We further showed that the increase of producers could be reinforced by both horizontal and vertical composition, as explained above.

3.4. Compositional and diversity effects

It should be noted that the compositional effects we report on here cannot be readily translated to diversity effects. In order to directly test for horizontal and vertical diversity effects, composition should be replicated within each diversity level, as done in biodiversity-ecosystem function research (De Laender et al., 2016). Instead, our design should be understood as a test of the effects of embedding a reference set of selected single producer and consumer species (*R. subcapitata* and *M. macrocopa* respectively) into a community of increasing complexity. While we selected this reference set randomly, future works could base this selection on occurrence frequency. *C. vulgaris* and *D. pulex*, for example, are dominant green alga and Cladocera in many natural lakes and ponds, and could therefore have been chosen as our reference set (Cohen and Post, 1993; Hassan and Alkam, 2008; Hebert and Finston, 2001; Steiner, 2002; Sze, 1980; Tiwari and Chauhan, 2006; Wen et al., 2005). Speculating how such an alternative selection of the reference set would have changed our results is not straightforward, as this will depend on both the sensitivity and the ecology of the species (Baert et al., 2017). When based solely on sensitivity arguments, we do not expect selecting this different reference set would have changed our results considerably. Indeed, toxicity data suggest that the consumers *D. pulex* and *M. macrocopa* have similar sensitivities

for the chlorpyrifos, as do the producers *R. subcapitata* and *C. vulgaris* herbicide linuron (Table S4). We further expect that, again based on sensitivity arguments only, our conclusions regarding composition effects will also be valid when we should have selected other species (i.e. the producer *S. acutus and* the herbivore *D. magna*), as their sensitivity is similar to that of our chosen reference set (Table S4).

5. Conclusions

Our experiment and analyses demonstrate that the direct and indirect effect of pesticides on aquatic ecosystems depends on horizontal and vertical composition. From these results, the following main conclusions can be drawn: (1) changing horizontal composition by adding species to our reference species increased or decreased the (in)direct effect of pesticides, depending on the type of pesticide used; (2) changing vertical composition by adding trophic levels always made (in)direct effects larger, regardless of the type of pesticide used. One important implication of our results is that the effects of pesticides on single species do not always correspond to worst-case scenarios and that protecting the most sensitive species does not protect the whole ecosystem. Given that community composition of natural systems widely varies between and within systems, we call for more research on how horizontal and vertical composition and diversity affect food-web resistance and resilience. Such studies will improve our understanding of the interaction between toxicological and ecological mechanisms, which is greatly needed to improve our understanding of the environmental impacts of chemicals and their risk assessment (Van den Brink et al., 2018).

Declaration of Competing Interest

The authors declare that we have no conflicts of interest to this work and there are no competing financial interests. We declare that all authors agreed to the contents in manuscript.

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References

Anderson, J.S., Teutsch, M., Dong, Z., Delgado-lo, F., Nu-, F., Vera, J.C., Hajra, A., Schro, E., Ried, T., Liu, P.P., Collins, F.S., 1996. A meta-analysis of the freshwater trophic cascade. Proc. Natl. Acad. Sci. 93, 7723–7726. https://doi.org/10.1073/pnas.93.15.7723

Artigas, J., Arts, G., Babut, M., Caracciolo, A.B., Charles, S., Chaumot, A., Combourieu, B., Dahllöf, I., Despréaux, D., Ferrari, B., Friberg, N., Garric, J., Geffard, O., Gourlay-Francé, C., Hein, M., Hjorth, M., Krauss, M., De Lange, H.J., Lahr, J., Lehtonen, K.K., Lettieri, T., Liess, M., Lofts, S., Mayer, P., Morin, S., Paschke, A., Svendsen, C., Usseglio-Polatera, P., Van den Brink, N., Vindimian, E., Williams, R., 2012. Towards a renewed research agenda in ecotoxicology. Environ. Pollut. 160, 201–206. https://doi.org/10.1016/j.envpol.2011.08.011

Baert, J.M., De Laender, F., Janssen, C.R., 2017. The Consequences of Nonrandomness in Species-Sensitivity in Relation to Functional Traits for Ecosystem-Level Effects of Chemicals. Environ. Sci. Technol. 51, 7228–7235. https://doi.org/10.1021/acs.est.7b00527

Baert, J.M., De Laender, F., Sabbe, K., Janssen, C.R., 2016. Biodiversity increases functional and compositional resistance, but decreases resilience in phytoplankton communities. Ecology 97, 3433–3440. https://doi.org/10.1002/ecy.1601

Becker, J.M., Liess, M., 2017. Species Diversity Hinders Adaptation to Toxicants. Environ. Sci. Technol. 51, 10195–10202. https://doi.org/10.1021/acs.est.7b02440

Beketov, M.A., Liess, M., 2006. The influence of predation on the chronic response of Artemia sp. populations to a toxicant. J. Appl. Ecol. 43, 1069–1074. https://doi.org/10.1111/j.1365-2664.2006.01226.x

Black, A.R., Dodson, S.I., 1990. Demographic costs of Chaoborus-induced phenotypic

plasticity in Daphnia pulex. Oecologia 83, 117–122. https://doi.org/10.1007/BF00324642
Bracewell, S., Verdonschot, R.C.M., Schäfer, R.B., Bush, A., Lapen, D.R., Van den Brink, P.J., 2019. Qualifying the effects of single and multiple stressors on the food web structure of Dutch drainage ditches using a literature review and conceptual models. Sci. Total Environ. 684, 727–740. https://doi.org/10.1016/j.scitotenv.2019.03.497
Brock, T.C., Arts, G.H., Maltby, L., Van den Brink, P.J., 2006. Aquatic risks of pesticides, ecological protection goals, and common aims in European Union Legislation. Integr. Environ. Assess. Manag. 2, e20–e46. https://doi.org/10.1002/ieam.5630020402
Campero, M., Slos, S., Ollevier, F., Stoks, R., 2007. Sublethal pesticide concentrations and predation jointly shape life history: Behavioral and physiological mechanisms. Ecol. Appl. 17, 2111–2122. https://doi.org/10.1890/07-0442.1
Clements, W.H., Rohr, J.R., 2009. Community responses to contaminants: using basic ecological principles to predict ecotoxicological effects. Environ. Toxicol. Chem. 28, 1789–1800. https://doi.org/10.1897/09-140.1
Cohen, I., Post, A.F., 1993. The heterotrophic connection in a photoautotrophic chlorella vulgaris dominant in wastewater oxidation ponds. Water Sci. Technol. 27, 151–155. https://doi.org/10.2166/wst.1993.0546
Crane, M., Maycock, D., Watts, C.D., Atkinson, C., Johnson, I., 2007. Proposed EQS for Water Framework Directive Annex VIII substances: 2,4- dichlorophenol.
Cuppen, J.G.M., Van den Brink, P.J., Hartgers, E.M., Fettweis, U., Crum, S.J.H., Van Donk, E., Brock, T.C.M., 1997. Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. Ecotoxicol. Environ. Saf. 38, 13–24. https://doi.org/10.1006/eesa.1997.1555
Daam, M.A., Rodrigues, A.M.F., Van den Brink, P.J., Nogueira, A.J.A., 2009. Ecological effects of the herbicide linuron in tropical freshwater microcosms. Ecotoxicol. Environ. Saf. 72, 410–423. https://doi.org/10.1016/j.ecoenv.2008.07.009
Daam, M.A., Van den Brink, P.J., 2007. Effects of chlorpyrifos, carbendazim, and linuron on the ecology of a small indoor aquatic microcosm. Arch. Environ. Contam. Toxicol. 53, 22–35. https://doi.org/10.1007/s00244-006-0001-y
Daam, M.A., Van den Brink, P.J., Nogueira, A.J.A., 2008. Impact of single and repeated applications of the insecticide chlorpyrifos on tropical freshwater plankton communities. Ecotoxicology 17, 756–771. https://doi.org/10.1007/s10646-008-0227-8
Darbro, J.M., Johnson, P.H., Thomas, M.B., Ritchie, S.A., Kay, B.H., Ryan, P.A., 2012. Effects of Beauveria bassiana on survival, blood-feeding success, and fecundity of Aedes aegypti in laboratory and semi-field conditions. Am. J. Trop. Med. Hyg. 86, 656–664. https://doi.org/10.4269/ajtmh.2012.11-0455
De Laender, F., Janssen, C.R., 2013. Brief communication: The ecosystem perspective in ecotoxicology as a way forward for the ecological risk assessment of chemicals. Integr. Environ. Assess. Manag. 9, e34–e38. https://doi.org/10.1002/ieam.1428
De Laender, F., Morselli, M., Baveco, H., Van den Brink, P.J., Di Guardo, A., 2015. Theoretically exploring direct and indirect chemical effects across ecological and exposure scenarios using mechanistic fate and effects modelling. Environ. Int. 74, 181–190. https://doi.org/10.1016/j.envint.2014.10.012
De Laender, F., Rohr, J.R., Ashauer, R., Baird, D.J., Berger, U., Eisenhauer, N., Grimm, V., Hommen, U., Maltby, L., Meliàn, C.J., Pomati, F., Roessink, I., Radchuk, V., Van den Brink, P.J., 2016. Reintroducing Environmental Change Drivers in Biodiversity–

493 Ecosystem Functioning Research. Trends Ecol. Evol. 31, 905–915.
494 https://doi.org/10.1016/j.tree.2016.09.007
495 Duffy, J.E., Cardinale, B.J., France, K.E., McIntyre, P.B., Thébault, E., Loreau, M., 2007. The
496 functional role of biodiversity in ecosystems: Incorporating trophic complexity. Ecol.
497 Lett. 10, 522–538. https://doi.org/10.1111/j.1461-0248.2007.01037.x
498 Duffy, J.E., Paul, J., Elizabeth, A., 2003. Grazer diversity effects on ecosystem functioning in
499 seagrass beds. Ecol. Lett. 6, 637–645.
500 Fleeger, J.W., Carman, K.R., Nisbet, R.M., 2003. Indirect effects of contaminants in aquatic
501 ecosystems. Sci. Total Environ. 317, 207–233. https://doi.org/10.1016/S0048-
502 9697(03)00141-4
503 Gonzalez, A., Loreau, M., 2009. The Causes and Consequences of Compensatory Dynamics in
504 Ecological Communities. Annu. Rev. Ecol. Evol. Syst. 40, 393–414.
505 https://doi.org/10.1146/annurev.ecolsys.39.110707.173349
506 Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J., Tilman, D., 2011. Plant diversity and
507 the stability of foodwebs. Ecol. Lett. 14, 42–46. https://doi.org/10.1111/j.1461-
508 0248.2010.01548.x
509 Halstead, N.T., Mcmahon, T.A., Johnson, S.A., Raffel, T.R., Romansic, J.M., Crumrine, P.W.,
510 Rohr, J.R., 2014. Community ecology theory predicts the effects of agrochemical
511 mixtures on aquatic biodiversity and ecosystem properties. Ecol. Lett. 17, 932–941.
512 https://doi.org/10.1111/ele.12295
513 Hassan, F.M., Alkam, F., 2008. Phytoplankton and related nutrients in sawa lake, iraq. J.
514 Dohuk Univ. 11, 67–76. https://doi.org/10.1016/B978-0-12-410416-7.00009-4
515 Hebert, P.D.N., Finston, T.L., 2001. Macrogeographic patterns of breeding system diversity in
516 the Daphnia pulex group from the United States and Mexico. Heredity (Edinb). 87, 153–
517 161. https://doi.org/10.1046/j.1365-2540.2001.00885.x
518 Hebert, P.D.N., Grewe, P.M., 1985. Chaoborus-induced shifts in the morphology of Daphnia
519 ambigua. Limnol. Oceanogr. 30, 1291–1297. https://doi.org/10.4319/lo.1985.30.6.1291
520 Horn, W., 1991. The influence of biomass and structure of the crustacean plankton on the
521 water transparency in the Saidenbach storage reservoir. Hydrobiologia 225, 115–120.
522 https://doi.org/10.1007/BF00028390
523 Janssens, L., Stoks, R., 2017. Chlorpyrifos-induced oxidative damage is reduced under
524 warming and predation risk: Explaining antagonistic interactions with a pesticide.
525 Environ. Pollut. 226, 79–88. https://doi.org/10.1016/j.envpol.2017.04.012
526 Janssens, L., Stoks, R., 2013. Synergistic effects between pesticide stress and predator cues:
527 Conflicting results from life history and physiology in the damselfly Enallagma
528 cyathigerum. Aquat. Toxicol. 132–133, 92–99.
529 https://doi.org/10.1016/j.aquatox.2013.02.003
530 Kersting, K., van Wijngaarden, R., 1992. Effects of chlorpyrifos on a microecosystem. Environ.
531 Toxicol. Chem. 11, 365–372. https://doi.org/10.1002/etc.5620110310
532 Na, W., Lili, L., Kaifeng, S., Shunshan, D., 2012. Analysis of structure -activity relationship and
533 toxicity of organophosphorus pesticide to plankton. Ecol. Environ. Sci. 21, 118–123.
534 Naeem, S., Li, S., 1998. Consumer species richness and autotrophic biomass. Ecology 79,
535 2603–2615. https://doi.org/10.1890/0012-9658(1998)079[2603:CSRAAB]2.0.CO;2
536 Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., 1999. Trophic cascades revealed in diverse
537 ecosystems. Trends Ecol. Evol. 14, 483–488.
538 Preston, B.L., 2002. Indirect effects in aquatic ecotoxicology: Implications for ecological risk
539 assessment. Environ. Manage. 29, 311–323. https://doi.org/10.1007/s00267-001-0023-

1
Relyea, R.A., Mills, N., 2001. Predator-induced stress makes the pesticide carbaryl more deadly to gray treefrog tadpoles (Hyla versicolor). Proc. Natl. Acad. Sci. U. S. A. 98, 2491–2496. https://doi.org/10.1073/pnas.031076198
Rohr, J.R., Crumrine, P.W., 2005. Effects of an herbicide and an insecticide on pond community structure and processes. Ecol. Appl. 15, 1135–1147. https://doi.org/10.1890/03-5353
Rohr, J.R., Kerby, J.L., Sih, A., 2006. Community ecology as a framework for predicting contaminant effects. Trends Ecol. Evol. 21, 606–613. https://doi.org/10.1016/j.tree.2006.07.002
Rohr, J.R., Salice, C.J., Nisbet, R.M., 2016. The pros and cons of ecological risk assessment based on data from different levels of biological organization. Crit. Rev. Toxicol. 46, 756–784. https://doi.org/10.1080/10408444.2016.1190685
Rubach, M.N., Crum, S.J.H., Van den Brink, P.J., 2011. Variability in the dynamics of mortality and immobility responses of freshwater arthropods exposed to chlorpyrifos. Arch. Environ. Contam. Toxicol. 60, 708–721. https://doi.org/10.1007/s00244-010-9582-6
Slijkerman, D.M.E., Moreira-Santos, M., Jak, R.G., Ribeiro, R., Soares, A.M.V.M., Van Straalen, N.M., 2005. Functional and structural impact of linuron on a freshwater community of primary producers: The use of immobilized algae. Environ. Toxicol. Chem. 24, 2477–2485. https://doi.org/10.1897/04-658R.1
Snel, J.F.H., Vos, J.H., Gylstra, R., Brock, T.C.M., 1998. Inhibition of photosystem II (PSII) electron transport as a convenient endpoint to assess stress of the herbicide linuron on freshwater plants. Aquat. Ecol. 32, 113–123. https://doi.org/10.1023/A:1009971930626
Srivastava, D.S., Bell, T., 2009. Reducing horizontal and vertical diversity in a foodweb triggers extinctions and impacts functions. Ecol. Lett. 12, 1016–1028. https://doi.org/10.1111/j.1461-0248.2009.01357.x
Steiner, C.F., 2002. Context-dependent effects of Daphnia pulex on pond ecosystem function: Observational and experimental evidence. Oecologia 131, 549–558. https://doi.org/10.1007/s00442-002-0934-4
Stephenson, R.R., Kane, D.F., 1984. Persistence and Effects of Chemicals in Small Enclosures in Ponds R. Arch. Environ. Contam. Toxicol. 13, 313–326.
Sze, P., 1980. Seasonal succession of phytoplankton in Onondaga Lake, New York (USA). Phycologia 19, 54–59.
Tiwari, A., Chauhan, S.V.S., 2006. Seasonal phytoplanktonic diversity of Kitham lake, Agra. J. Environ. Biol. 27, 35–38.
Tollrian, R., 1993. Neckteeth formation in Daphnia pulex as an example of continuous phenotypic plasticity: morphological effects of Chaoborus kairomone concentration and their quantification. J. Plankton Res. 15, 1309–1318. https://doi.org/10.1093/plankt/15.11.1309
Trekels, H., Van de Meutter, F., Stoks, R., 2013. Predator cues magnify effects of the pesticide endosulfan in water bugs in a multi-species test in outdoor containers. Aquat. Toxicol. 138–139, 116–122. https://doi.org/10.1016/j.aquatox.2013.04.008
Trekels, H., Van de Meutter, F., Stoks, R., 2011. Effects of species-specific interactions with predation risk on the relative species sensitivities to a pesticide in water boatmen (Corixidae). Oikos 120, 897–905. https://doi.org/10.1111/j.1600-0706.2010.18852.x
USEPA, 2020. Sustainable Materials Management: The Road Ahead;U.S., Environmental Protection Agency: Washington, DC, June 2009. https://doi.org/10.1021/es202079y

Van den Brink, P.J., Boxall, A.B.A., Maltby, L., Brooks, B.W., Rudd, M.A., Backhaus, T., Spurgeon, D., Verougstraete, V., Ajao, C., Ankley, G.T., Apitz, S.E., Arnold, K., Brodin, T., Cañedo-Argüelles, M., Chapman, J., Corrales, J., Coutellec, M.A., Fernandes, T.F., Fick, J., Ford, A.T., Giménez Papiol, G., Groh, K.J., Hutchinson, T.H., Kruger, H., Kukkonen, J.V.K., Loutseti, S., Marshall, S., Muir, D., Ortiz-Santaliestra, M.E., Paul, K.B., Rico, A., Rodea-Palomares, I., Römbke, J., Rydberg, T., Segner, H., Smit, M., van Gestel, C.A.M., Vighi, M., Werner, I., Zimmer, E.I., van Wensem, J., 2018. Toward sustainable environmental quality: Priority research questions for Europe. Environ. Toxicol. Chem. 37, 2281–2295. https://doi.org/10.1002/etc.4205

Van den Brink, P.J., Hartgers, E.M., Fettweis, U., Crum, S.J.H., Van Donk, E., Brock, T.C.M., 1997. Sensitivity of macrophyte-dominated freshwater microcosms to chronic levels of the herbicide linuron. Ecotoxicol. Environ. Saf. 38, 13–24. https://doi.org/10.1006/eesa.1997.1555

Van den Brink, P.J., Klein, S.L., Rico, A., 2017. Interaction between stress induced by competition, predation, and an insecticide on the response of aquatic invertebrates. Environ. Toxicol. Chem. 36, 2485–2492. https://doi.org/10.1002/etc.3788

Van den Brink, P.J., Van Wijngaarden, R.P.A., Lucassen, W.G.H., Brock, T.C.M., Leeuwangh, P., 1996. Effects of the insecticide Dursban® 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. Environ. Toxicol. Chem. 15, 1143–1153. https://doi.org/10.1002/etc.5620150719

Van Wijngaarden, R.P.A., Brock, T.C.M., Douglas, M.T., 2005. Effects of chlorpyrifos in freshwater model ecosystems: The influence of experimental conditions on ecotoxicological thresholds. Pest Manag. Sci. 61, 923–935. https://doi.org/10.1002/ps.1084

Wang, S., Brose, U., 2018. Biodiversity and ecosystem functioning in food webs: the vertical diversity hypothesis. Ecol. Lett. 21, 9–20. https://doi.org/10.1111/ele.12865

Wen, Z., Mian-Ping, Z., Xian-Zhong, X., Xi-Fang, L., Gan-Lin, G., Zhi-Hui, H., 2005. Biological and ecological features of saline lakes in northern Tibet, China. Hydrobiologia 541, 189–203. https://doi.org/10.1007/s10750-004-5707-0

Zhao, Q., Van, P.J. den B., Carpentier, C., Wang, Y.X.G., Rodrıguez-Sanchez, P., Xu, C., Vollbrecht, S., Gillissen, F., Vollebregt, M., Wang, S., Laender, F. De, 2019. Horizontal and vertical diversity jointly shape food web stability against small and large perturbations. Ecol. Lett. 22, 1152–1162. https://doi.org/10.1111/ele.13282

Figures

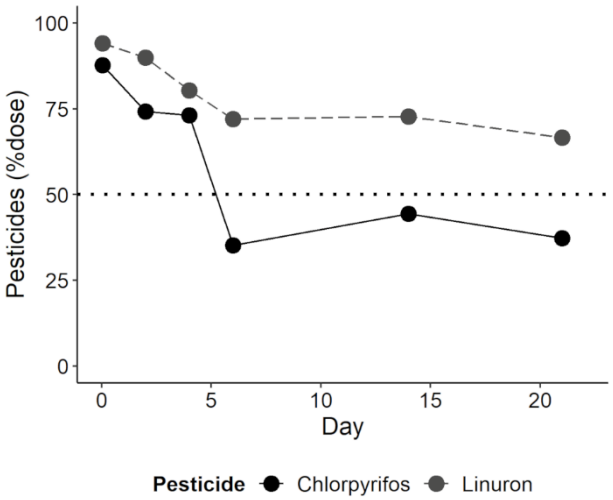


Figure 1. Concentration of linuron and chlorpyrifos in systems on sample 1h and day 2, 4, 6, 14 and 21.

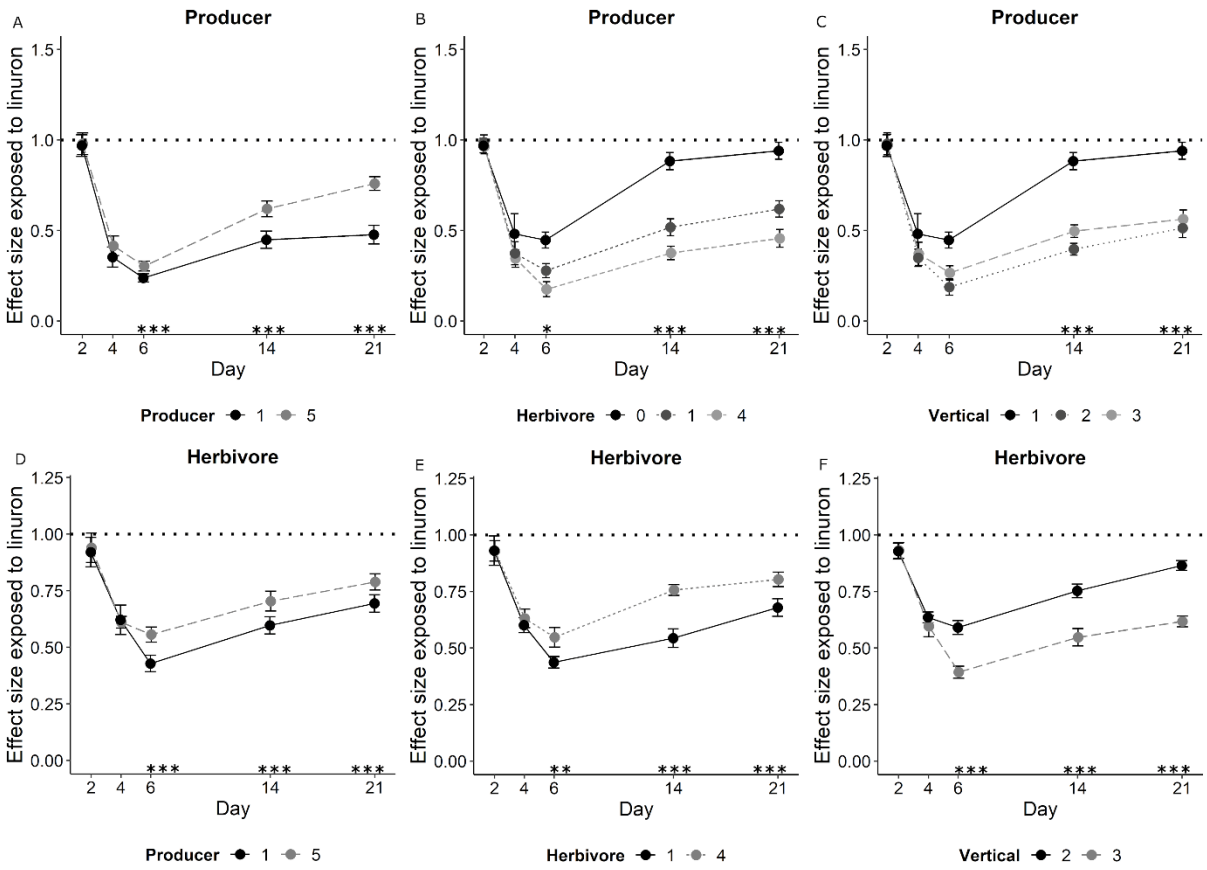


Figure 2. The effects of horizontal composition of producer and herbivore, and vertical composition on effect sizes (biovolume as proxy) of producers (A-C), herbivores (D-F) after exposure to linuron. Plotted are sample mean ± 1 SE. An effect size is 1 (treatment = control) indicating no effect of linuron, smaller than 1 (treatment < control) indicating a negative effect of linuron, and bigger than 1 (treatment > control) indicating a positive impact. The bigger deviation from effect size 1 (dash line) indicates larger effect of linuron. The effect size with 1 and 5 producers (A and D) was visualized by averaging effect sizes of all treatments with 1 and 5 producers, respectively, similar manipulation for the effect size under 0, 1 and 4 herbivores species (B and E) and for the effect size under 1, 2 and 3 vertical composition (C and F). Detailed statistical results are listed in Table S5.1-S5.2. (*P< 0.05, ** P < 0.01, *** P < 0.001).

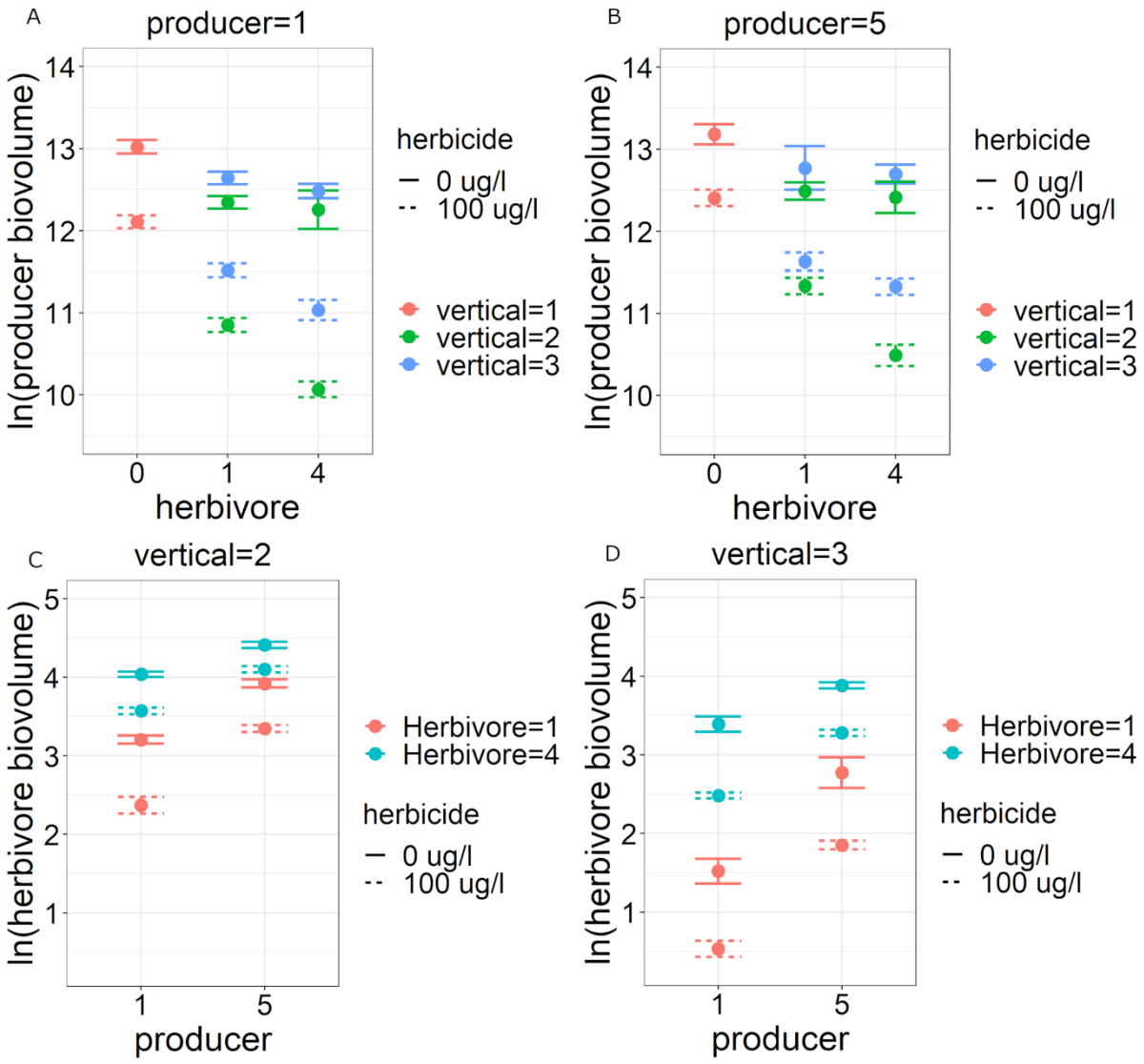
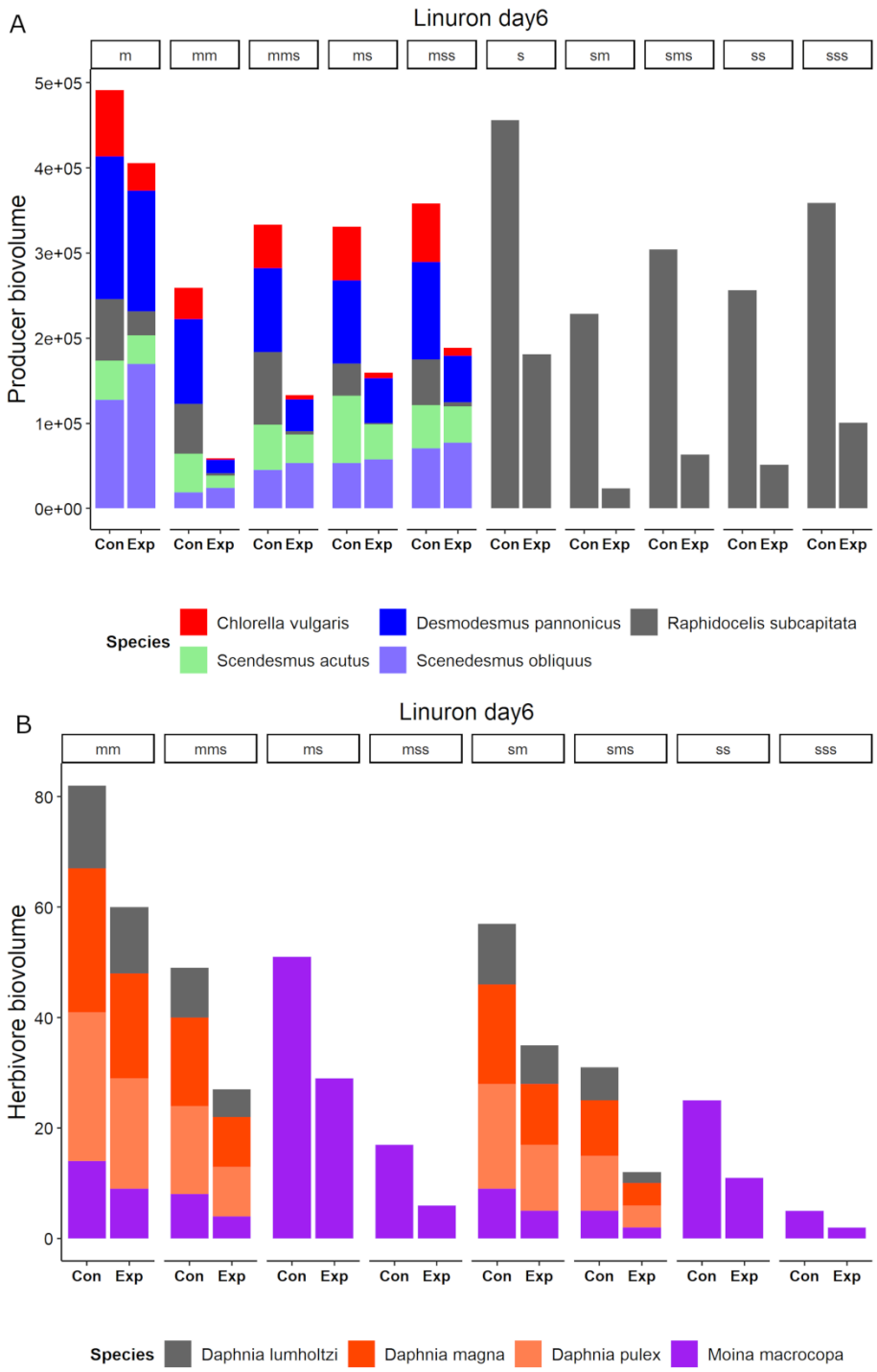


Figure 3. The interactive effects of horizontal (producers and herbivores) and vertical composition, herbicide linuron on ln(producer biovolume) (a, b) and on ln(herbivore biovolume) (c, d). Plotted are sample mean ± 1 SE. Solid error bars indicate linuron concentration of 0 µg l^{-1}, while dashed ones stand for linuron concentration of 100 µg l^{-1}. Detailed statistical results are listed in Table S6.



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655 **Figure 4.** Species biovolume in the ten community types after linuron exposure on day 6. Con
656 represents control group, and Exp stands for exposure. Ten treatments include: *s*, single algae;
657 *ss*, single algae-single herbivore; *sss*, single algae-single herbivore-single predator; *sm*, single
658 algae-multiple herbivores; *sms*, single algae-multiple herbivores-single predator; *m*, multiple
659 algae; *ms*, multiple algae-single herbivore; *mss*, multiple algae-single herbivore-single
660 predator; *mm*, multiple algae-multiple herbivores; *mms*, multiple algae-multiple herbivores-
661 single predator). Detailed statistical results are listed in Table S7.1-7.2.

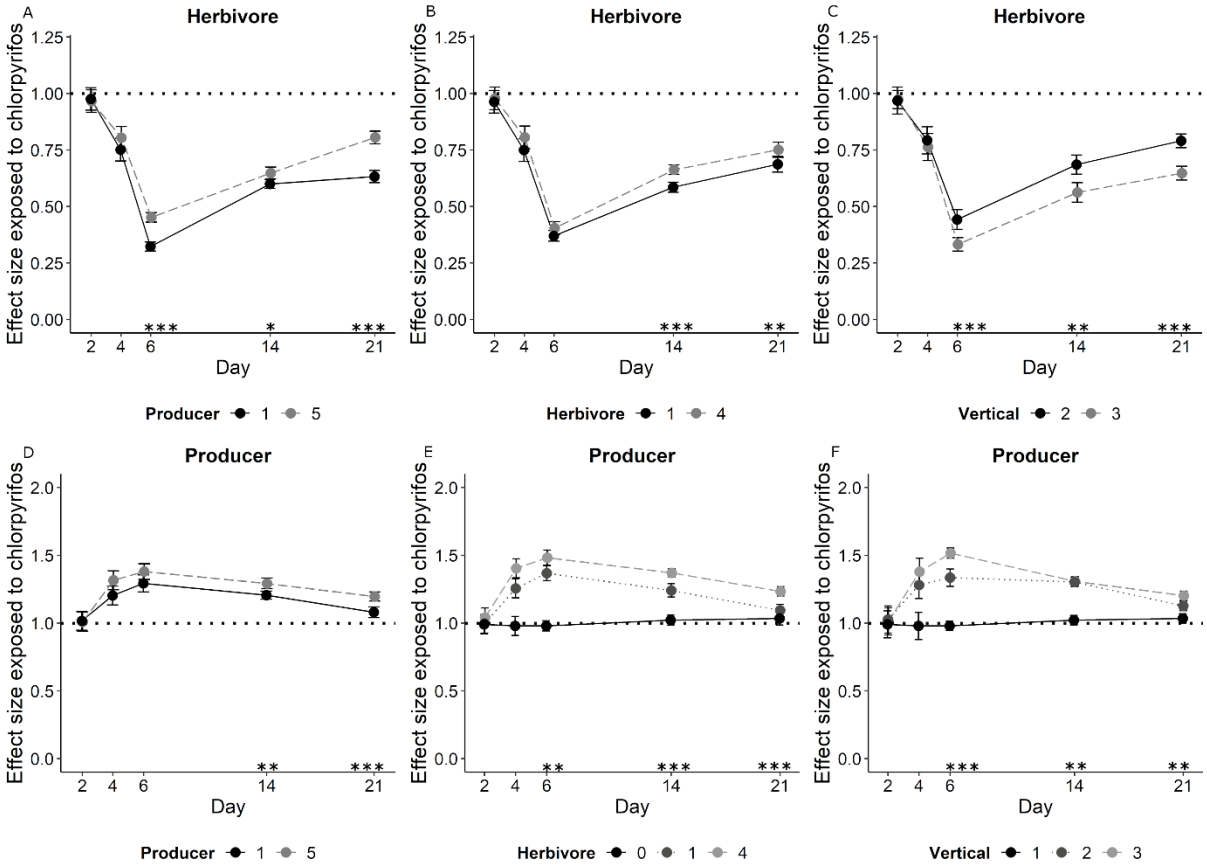


Figure 5. The effects of horizontal composition of producer and herbivore, and vertical composition on effect sizes (biovolume as proxy) for herbivores (A-C), producers (D-F) after exposure to chlorpyrifos. Plotted are sample mean ± 1 SE. An effect size is 1 (treatment = control) indicating no effect of chlorpyrifos, smaller than 1 (treatment < control) indicating a negative effect of chlorpyrifos, and bigger than 1 (treatment > control) indicating a positive impact. The effect sizes with 1 and 5 produces (A and D) was visualized by averaging effect size of all treatments with 1 and 5 producers, respectively, similar manipulation for the effect size under 0, 1 and 4 herbivores (B and E) and for the effect size under 1, 2 and 3 vertical composition (C and F). The bigger deviation from effect size 1 (dash line) indicates larger effect of chlorpyrifos. Detailed statistical results are listed in Table S8.1-S8.2. (*P< 0.05, ** P < 0.01, *** P < 0.001).

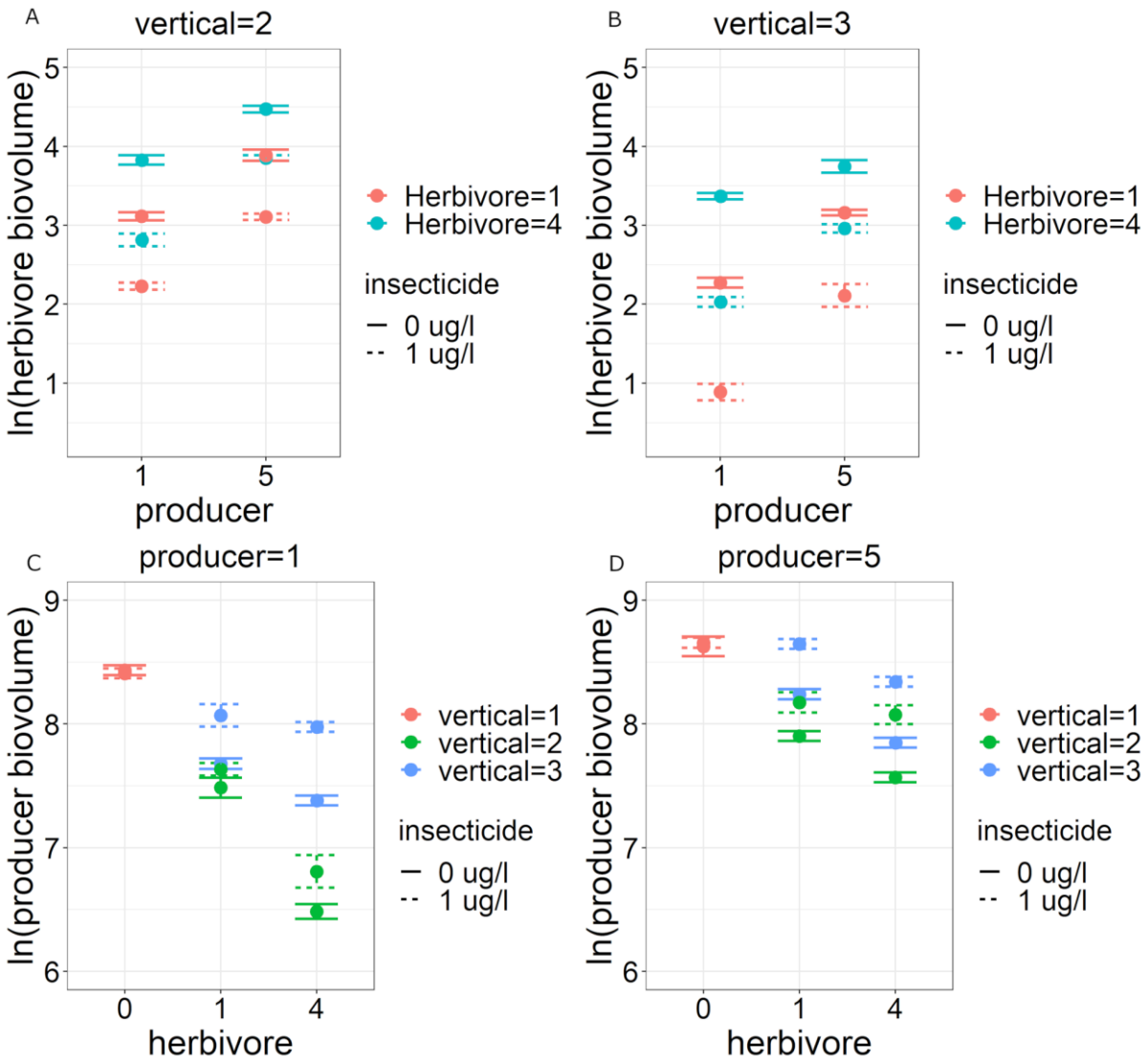
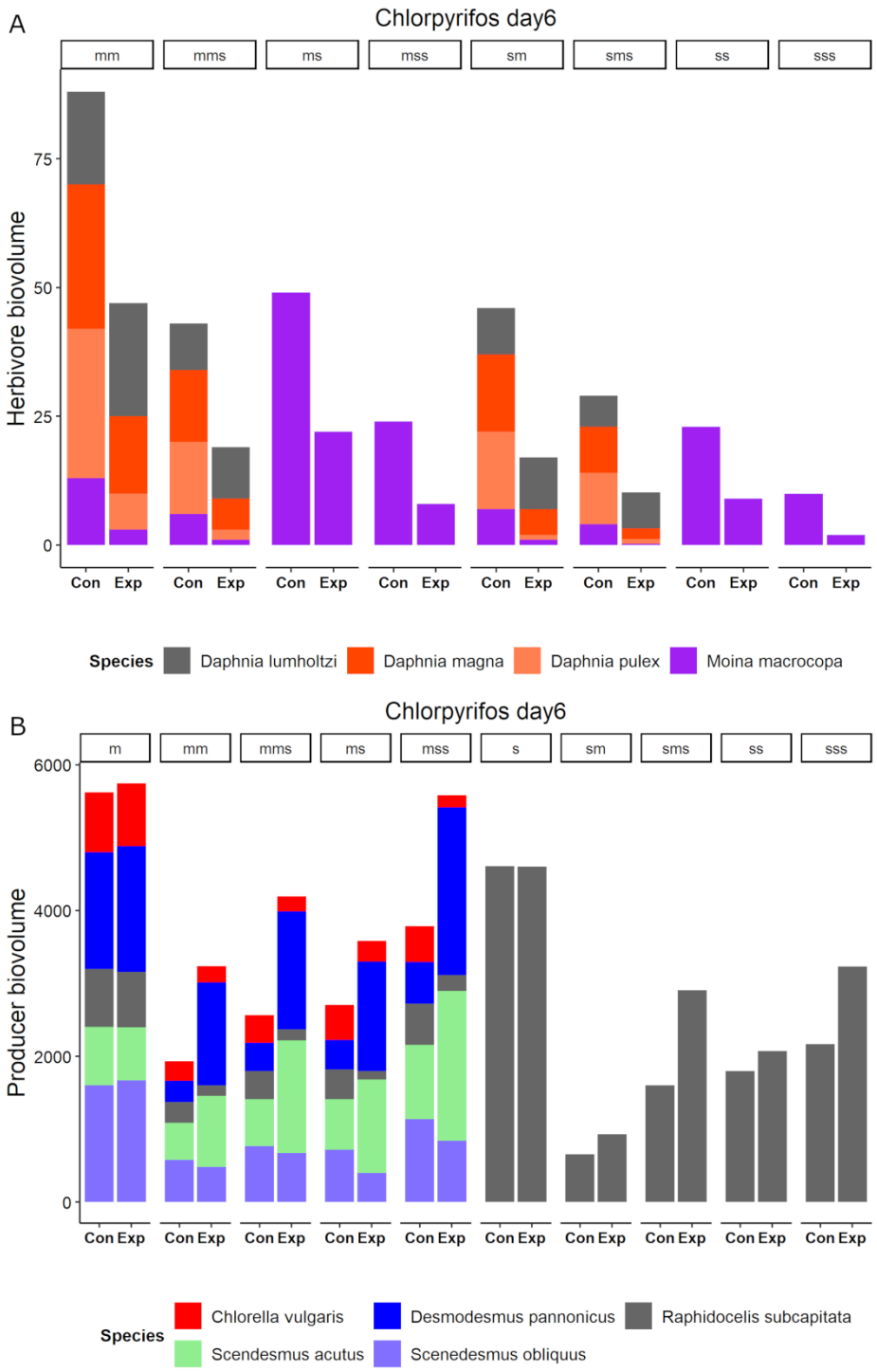


Figure 6. The interactive effects of horizontal (producer and herbivore) and vertical composition, insecticide chlorpyrifos on ln(herbivore biovolume) (a, b) and on ln(producer biovolume) (c, d). Plotted are sample mean ± 1 SE. Solid error bars indicate chlorpyrifos concentration of 0 µg l^{-1}, while dashed ones stand for chlorpyrifos concentration of 1 µg l^{-1}. Detailed statistical results are listed in Table S6.



683

684 **Figure 7.** Species absolute biovolume in the ten community types after chlorpyrifos exposure

685 on day 6. Con represents control group, and Exp stands for exposure. Ten treatments are same

686 as Figure 4. Detailed statistical results are listed in Table S9.1-9.2.

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